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Seasonal Acclimatization to Temperature in Cardueline Finches

II. Changes in Body Composition and Mass in Relation to Season and Acute Cold Stress

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Summary. 1. Seasonal variation in body constituents and utilization of lipid, protein, and carbohydrate during cold stress in American goldfinches were studied to determine relations of these functions to the pronounced seasonal shift in thermogenic capacity documented in a previous study (Dawson and Carey, 1976).

2. Mean body mass for adults increases from a low of 11.4 g in July to a high of 15.1 g in December and January. Seasonal variation in lipid content accounts for the major part of the observed changes in body mass, but such variation in water and protein content is also appreciable.

3. Linoleic acid (18:2) is the predominant fatty acid in neutral lipids of liver, pectoralis muscle, and furcular depots at all seasons. Unsaturated fatty acids comprise a much greater proportion of total fatty acids in liver and pectoralis muscle during winter (71% and 73%, respectively) than in spring or fall.

4. Fasting winter goldfinches exposed to −10 °C for 17 h overnight utilize significant amounts of body lipid. However, total body protein, liver and pectoralis muscle carbohydrate, and pectoralis muscle fatty acids do not differ significantly between control and cold-stressed individuals.

5. Glycogen stores in the pectoralis muscles are significantly higher in winter than in summer birds. Winter goldfinches exposed to −70 °C utilize significant amounts of total body lipids and pectoralis glycogen. Birds tested in this manner in summer do not do so and quickly become hypothermic.

6. Histochemical characteristics and succinate oxidase activities of pectoralis muscles do not vary appreciably over the year.

7. Increased stores of body lipid and muscle carbohydrate and the ability to mobilize these substrates rapidly during cold stress seem to be key factors in the superior thermogenic capacities of winter goldfinches.

Introduction

The interacting problems of cold, short days, and diminishing food supplies confronting small birds wintering in northern areas appear to pose severe challenges to thermoregulation and maintenance of energy balance. Cold increases the requirements of these diurnally feeding animals for energy and nutrients at a time when the daily periods in which to obtain food are most restricted. The possible importance of seasonal acclimatization in enhancing the capacities of small birds for dealing with winter conditions has led us to examine this phenomenon in one of the more prominent avian groups of boreal regions, the cardueline finches. Our initial report on seasonal acclimatization of one representative of this group, the American goldfinch (*Carduelis* [*Spinus*] *tristis*), to temperature (Dawson and Carey, 1976) established that heightened thermogenic capacity, rather than increased insulation, constituted the major feature of their adjustment to winter conditions. Goldfinches quickly became hypothermic when exposed to severe cold (−70 °C) in spring, summer, and fall, yet displayed remarkable powers of thermoregulation under such conditions in winter. This increased thermoregulatory capacity was mainly dependent on an ability to attain and sustain adequate heat production.

We now consider more closely this seasonally enhanced thermogenic ability in American goldfinches by examining seasonal changes in body composition, patterns of substrate utilization under moderate and severe cold stress, and oxidative capacities of skeletal muscle at various times of the year.

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We used animals freshly obtained from the field in our study, so that we could analyze most effectively the natural patterns and processes of seasonal acclimatization in these small birds.

Materials and Methods

Experimental Animals. American goldfinches are present in southern Michigan throughout the year. The birds used in this study were obtained from flocks near Ann Arbor, Washtenaw County, Michigan in 1972–1976. Goldfinches were captured by mist net in the spring, summer, and fall and by trap in the winter. Most birds were caught before 1200 EST.

Seasonal Variation of Body Mass. Birds were taken to the laboratory immediately after capture and weighed to the nearest 0.1 g on a Mettler top-loading balance. Age, sex, and stage of molt were determined from plumage characteristics. The presence of an incubation patch or cloacal protuberance was noted.

Seasonal Variation of Body Constituents. Goldfinches were sacrificed by dislocation of the neck soon after arrival in the laboratory. The carcasses were weighed to the nearest 0.1 mg on a Mettler H20T balance. Contour plumage was carefully plucked from the head, neck, and body and dried to a constant mass (to the nearest mg) in an oven at 80 °C. Meanwhile, the carcass and attached flight feathers were lyophilized to constant mass. The differences between the initial body mass and the combined mass of a) the dry carcass and flight feathers plus b) the contour feathers represented the initial body water content. The dry carcass and flight feathers were cut into small pieces and introduced in a cellulose extraction thimble into a Soxhlet extraction apparatus. Neutral lipid was then extracted over 24 h by petroleum ether, after which the lean carcass was redried to constant mass at 80 °C. The difference between the masses of the carcass in the dry and lean dry conditions represented the neutral lipid portion of the original body mass. Protein content of the lean dry carcass and flight feathers was then determined. The material was dissolved completely over 24 h in 100 ml of 1N NaOH and the pH of the resultant solution lowered to 13.2 by addition of concentrated HCl. This solution was then diluted to 1 liter with distilled water and thoroughly mixed. The protein content of triplicate 0.05-ml samples of the solution was measured as absorbance at 500 nm on a Zeiss PQM-3 spectrophotometer (Lowry et al., 1951). Values so obtained were converted to actual concentrations by comparison with standard solutions of egg albumin treated in the same manner as that just described. The difference in masses of protein and the lean dry body represents skeletal and other inorganic materials and carbohydrates.

Seasonal Variation in Fatty Acid Content of Neutral Lipids. Approximately 0.3-g samples of pectoralis muscle and of liver were excised from freshly sacrificed goldfinches and homogenized for 10 min in 10 ml of chloroform-methanol (3:1 V:V) solution containing 0.01% Santoquin (1, 2-dihydro-6-ethoxy-2,2,4-trimethyl quinolin) to serve as an antioxidant. The homogenate was filtered through glass wool into a 15-ml tube. Highly polar molecules were removed from the homogenate by gentle shaking following addition of 3 ml of distilled water. The chloroform-methanol layer was removed, rewashed with water, and introduced into a 40-cm column containing 15 g of activated silicic acid (100 mesh) that removed polar lipids, leaving neutral lipids (principally glycerides and fatty acids) in the eluate (Kates, 1972). The solution was followed through the column by 250 ml of chloroform. The total eluate, about 300 ml, was evaporated to 8 ml in a Büchi-Brinkmann rotoevaporator and then by passing dry nitrogen gas over the solution. This 8-ml-sample was stored at 5 °C under nitrogen in a teflon-capped culture tube. Twenty µl of 5.12 mM pentadecanoic acid (15:0) was added to an aliquot of this sample prior to evaporation to dryness under nitrogen. The esters were then methylated with borontrifluoride methanol (14% W:V) (Applied Science Laboratories). Chain lengths of these esters were determined on a Varian Aerograph gas chromatograph (model 2400) with a 20% DEGS column maintained at 180 °C. The equivalent chain length method of Hofstetter et al. (1965) was used to identify individual fatty acids. Estimation of the total amount of fatty acids in each aliquot was done by the triangulation method of Brandt and Lands (1968).

Utilization of Substrates during Overnight Exposure to −10 °C. The decreases of lipids, proteins, and carbohydrates during overnight fasting by goldfinches at −10 °C indicated the relative importance of these materials as substrates for thermogenesis. Goldfinches were trapped between 0800 and 1300 h in January and February, 1976, and held undisturbed at 25 °C with ample water and a mixture of thistle and millet seeds. The birds were weighed at 1700 h and 13.5- to 15.5-g individuals selected for the following measurements. Twelve of these birds were sacrificed at 1700 h to serve as controls. Masses of their livers and pectoralis muscles were then determined. Six of the control birds were plucked and subjected to lyophilization, lipid extraction, and protein determination with procedures previously outlined. Samples of liver and pectoralis muscle from the other 6 controls were blotted dry, and quickly weighed with an H-14 Mettler balance. Portions of these tissues were placed in separate test tubes and rapidly dissolved in 2 ml of hot, 30% KOH. The carbohydrate, principally glycogen, in these mixtures was precipitated with 2 ml of 95% ethanol and then analyzed according to the anthrone method of Seifter et al. (1950). Another portion of pectoralis muscle was weighed, placed in a small polyethylene test tube, frozen in an acetone-dry ice bath, and then stored at −20 °C for later analysis of the fatty acid content by gas-liquid chromatography, as described above. The data obtained from these twelve birds provide estimates of the mean amounts of various substrates potentially available for use by the experimental group subjected to overnight fasting at −10 °C.

At 1700 h 12 experimental birds were placed without food in a Foster constant temperature cabinet in separate $0.3 \times 0.3 \times 0.3$ m cages covered on five sides with Saran wrap to reduce air movement. Snow was available as a water supply. Air temperatures were measured throughout the test by thermocouples connected to a Honeywell 15 multipoint recording potentiometer. The fluorescent lights in the Foster cabinet went off at 1730 h, close to the time of natural sunset, and turned on at 0830 h the next day. The birds were removed at 1000 h, having fasted 17 h at −10 °C. Following weighing, 6 were sacrificed for analysis of residual neutral lipid and total body protein, and 6 for pectoralis muscle glycogen and fatty acids and liver glycogen.

Substrate Utilization under Severe Cold Stress. The relative importance of lipid and carbohydrate as substrates for thermogenesis during severe cold stress in summer and winter birds was judged from the difference in amounts of these substances between birds acutely subjected to −70 °C and controls.

Goldfinches captured between 0900 and 1200 h in January-February and June-August served as "winter" and "summer" individuals respectively. Control birds were sacrificed immediately after arrival in the laboratory and the carcasses analysed for total body lipid or for pectoralis muscle and liver carbohydrate. Data obtained provide an estimate of the initial levels of such substrates available to birds in the experimental group. Experimental birds were used in the −70 °C experiments described previously (Dawson

and Carey, 1976) shortly after removal from the field. When such a bird could no longer sustain elevated levels of metabolism under the conditions in the test (as indicated by oxygen consumption starting to drop from peak levels attained at −70 °C), it was quickly removed and sacrificed for analysis of total body lipid or pectoralis muscle and liver carbohydrate.

Oxidative Capacity of Pectoralis Muscles at Various Seasons. Eight fall, five winter, four spring, and three summer goldfinches were sacrificed in the laboratory within two hours after capture. The heart and pectoralis muscles were excised and rinsed in ice cold physiological saline. The muscles were weighed, minced finely with scissors, and homogenized in ground glass homogenizing tubes containing ice cold 0.01 M phosphate buffer (39 ml/g muscle) at pH 7.4. Aliquots of the homogenates were assayed for succinate oxidase activity in a Gilson differential respirometer. A 2.3-ml volume of reaction medium (7.5 ml of 4 mM $AlCl_3$, 7.5 ml of 4 mM $CaCl_2$, 2.5 ml of 1 M phosphate buffer pH 7.4, 2.4 ml of 0.24 mM cytochrome c, 1.01 g sodium succinate, and 37.5 ml H_2O) was pipetted into the reaction chamber of each flask, 0.5 ml of homogenate was introduced into the side arm, and 0.2 ml of 15% KOH was placed in the center well which contained a filter paper wick to increase the absorptive surface for CO_2. Flasks were gassed with room air and equilibrated at 28 °C for 10 min. The homogenate was then tipped into the reaction chamber. At 5-min intervals, blank readings for flasks containing 0.5 ml of 0.01 M phosphate buffer in the side arm but otherwise treated the same as the experimental flasks were subtracted from the readings for the homogenates. Oxygen consumption was constant from 20 to 60 min of reaction and calculations of succinate oxidase activity were based on this interval. The data are expressed as µl (STPD) O_2 consumed $(\text{min}\cdot\text{g wet muscle})^{-1}$ and $(\text{min}\cdot\text{mg protein})^{-1}$. The protein content of the homogenates was determined by the method of Lowry et al. (1951).

Histochemistry. Small cubes of pectoralis muscle from three summer and three winter goldfinches were excised and quick-frozen in isopentane cooled with dry ice. Serial cross sections 10 µm thick were cut in a cryostat and mounted on cover glasses. Sections were incubated for succinic acid dehydrogenase (SDH) (Nachlas et al., 1957), myofibrillar ATPase (Chayen et al., 1972: 129–132), and capillary membrane phosphatase (Maxwell et al., 1977). Visualization of capillary membrane phosphatase was facilitated by fixation before incubation, in a solution of 1% $CaCl_2$ and 10% formalin (5 min) followed by rinsing in 0.2 M Tris-maleate (5 min). Sections incubated for SDH and myofibrillar ATPase activities were untreated prior to incubation. Sections were projected at 1000× magnification, outlines of 70–120 fibers from each muscle were traced, and fiber areas determined by planimetry. Each fiber was classified as to functional type on the basis of criteria developed for mammalian skeletal muscles by Maxwell et al. (1973). The number of capillaries adjacent to each fiber and per mm^2 were determined. The visualization of these vessels was made possible by the histochemical detection of the capillary membrane phosphatase.

Statistics. Unless otherwise specified, all statistical comparisons were made with Student's t-test, with significance accepted at $P<0.05$. One standard error is given with means.

Results

Body Mass

Adult American goldfinches in southern Michigan tend to be heaviest in December and January (mean body mass for both months, 15.1 g), but remain above 14 g through March. They are lightest during July, when they average approximately 11.4 g (Fig. 1, Table 1).

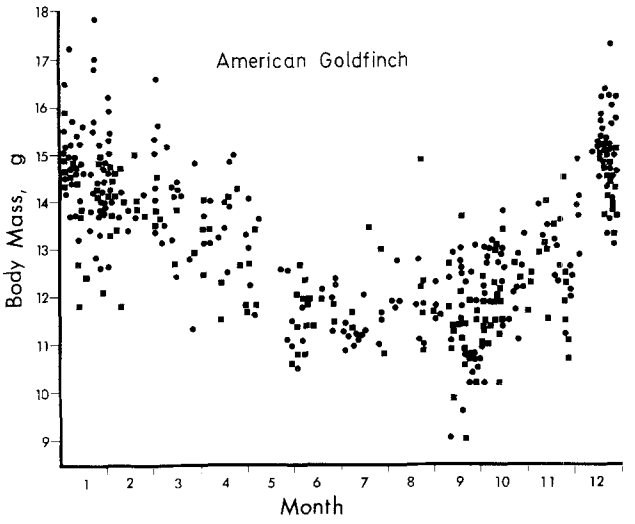

Fig. 1. Body masses of American goldfinches captured during the years of 1970–1975. Circles and squares represent males and females, respectively

Body masses of male, female and juvenil goldfinches are presented separately in Table 1. Adult males are heavier than their female counterparts during December ($P<0.02$) and January ($P<0.01$). The converse is true during July ($P<0.01$), presumably as a result of changes in the females associated with egg production (see next section). Adults are significantly ($P<0.01$) heavier than juveniles in both January and August.

Body Constituents

The values of principal body constituents for adults and young and for males and females have generally been pooled for determination of means; the amounts of these constituents usually did not differ significantly with age or gender, for sample sizes were small. The major exceptions were neutral lipid and protein contents of males and females during June and July (Table 2).

Neutral lipid shows conspicuous seasonal fluctuation. Goldfinches frequenting the Ann Arbor area accumulate this type of substrate rapidly during November. It reaches a maximum in December (1.67 ± 0.073 g). The mean fat contents for January and February birds (1.52 ± 0.097 g and 1.26 ± 0.173 g, respectively) do not differ significantly

Table 1. Mean (±S.E.M.) body masses (g) of American goldfinches. Numbers in parentheses are sample sizes. Juveniles were considered to be adults after the termination of pre-nuptial molt in March-April

Month	♂♂	♀♀	Adult	Juv.	Combined sample
Jan.	14.73±0.15 (49)	14.04±0.24 (19)	15.11±0.23 (26)	14.18±0.14 (42)	14.54±0.13 (68)
Feb.	14.40±0.24 (16)	13.99±0.20 (15)	14.24±0.28 (9)	14.19±0.19 (22)	14.20±0.16 (31)
Mar.	14.25±0.26 (21)	13.59±0.23 (7)	14.33±0.30 (17)	13.66±0.18 (11)	14.06±0.19 (28)
Apr.	13.77±0.24 (10)	13.15±0.32 (9)	13.48±0.20 (19)		13.48±0.20 (19)
May	12.42±0.26 (13)	11.89±0.55 (4)	12.29±0.24 (17)		12.29±0.24 (17)
June	11.84±0.23 (8)	11.97±0.13 (10)	11.91±0.12 (18)		11.91±0.12 (18)
July	11.27±0.09 (15)	12.28±0.64 (3)	11.44±0.15 (18)		11.44±0.15 (18)
Aug.	11.89±0.21 (9)	12.32±0.67 (5)	12.19±0.32 (11)	11.53±0.22 (3)	12.05±0.26 (14)
Sept.	11.77±0.17 (28)	11.42±0.25 (19)	12.00±0.16 (12)	11.51±0.18 (35)	11.63±0.14 (47)
Oct.	12.32±0.17 (25)	11.92±0.17 (21)	12.33±0.42 (2)	12.13±0.13 (44)	12.14±0.12 (46)
Nov.	13.07±0.21 (18)	12.46±0.24 (17)	12.98±0.36 (14)	12.64±0.18 (21)	12.78±0.17 (35)
Dec.	15.16±0.17 (31)	14.46±0.16 (15)	15.09±0.16 (12)	14.89±0.17 (34)	14.93±0.13 (46)

Table 2. Mean (±S.E.M.) body mass and body constituents of goldfinches captured in different months. Sample sizes for all means for each month are listed in parentheses. Data for each month are combined for sexes and ages except in June and July where males and females are presented separately

Month	Initial body					Water		
	Mass	Lipid	Protein[a]	Contour plumage	Residual[b]	Water	(% original body mass)	(% lean original body mass)
	g	g	g	g	g	g		
Jan. (12)	14.16±0.235	1.52 ±0.097	2.86±0.045	0.55±0.039	0.758±0.030	8.47±0.123	59.8±0.005	67.0±0.004
Feb. (7)	14.30±0.247	1.26 ±0.173	3.11±0.104	0.59±0.019	0.750±0.023	8.58±0.109	60.0±0.007	65.8±0.006
Mar. (14)	14.29±0.170	0.972±0.120	3.29±0.071	0.55±0.011	0.825±0.014	8.64±0.085	60.5±0.003	67.3±0.008
April (8)	13.23±0.229	0.665±0.086	2.92±0.089	0.52±0.029	0.772±0.043	8.34±0.181	63.1±0.007	66.3±0.006
May (8)	12.46±0.421	0.640±0.129	2.89±0.143	0.54±0.019	0.676±0.076	7.71±0.174	62.1±0.011	65.3±0.006
June ♂♂ (4)	11.71±0.263	0.347±0.085	2.47±0.058	0.46±0.031	0.781±0.026	7.65±0.138	65.3±0.005	67.3±0.002
June ♀ (1)	12.68	1.14	2.35	0.44	0.786	7.95	62.7	68.9
July ♂♂ (2)	11.36±0.303	0.314±0.003	2.47±0.055	0.38±0.025	0.800±0.010	7.39±0.211	65.0±0.002	66.9±0.001
July ♀ (1)	13.04	0.573	3.40	0.38	0.862	7.82	60.0	62.7
August (8)	11.87±0.237	0.486±0.062	2.57±0.094	0.38±0.030	0.687±0.049	7.74±0.180	65.2±0.004	68.0±0.005
Sept. (10)	11.46±0.309	0.331±0.060	2.59±0.128	0.32±0.019	0.626±0.038	7.59±0.163	66.3±0.006	68.2±0.005
Oct. (11)	12.16±0.226	0.344±0.050	2.72±0.095	0.44±0.045	0.690±0.034	7.95±0.158	65.4±0.006	67.3±0.005
Nov. (8)	12.04±0.234	0.733±0.221	2.49±0.059	0.50±0.178	0.772±0.027	7.55±0.346	62.6±0.023	66.5±0.030
Dec. (17)	15.07±0.213	1.67 ±0.073	2.95±0.058	0.59±0.164	0.792±0.014	9.05±0.130	60.0±0.002	67.5±0.002

[a] Protein includes total body protein and flight feathers. It excludes protein of contour feathers

[b] Residual includes masses of skeletal and other inorganic material and carbohydrates

from that for the December individuals. However, the March mean (0.98±0.120 g) marks a significant ($P<0.01$) decline in fat content from levels noted earlier in winter. Fat content of male birds continues to decline over the spring, reaching a minimal level in June that persists until October. Reproduction appears to influence the fat content of female goldfinches. *Carduelis tristis* in Michigan breed principally in July and August (Nickell, 1951). One female had 1.14 g of fat in June. Another measured in July had 0.57 g, nearly twice the average for males in this month (Table 2). Females appear as lean as males in late summer and early fall.

Other body constituents than neutral lipids also change in amount seasonally. Birds have more protein in winter and early spring than in late spring, summer, and November ($P<0.01$). This variation is paralleled by a similar trend in absolute body water content. Since fat contains little water relative to other tissues, its accumulation by winter birds reduces the proportion of water in the total body mass (Fig. 2).

The final category of body constituents defined in this study, includes carbohydrate stores, the skeletal system, and other inorganic materials, with the skeleton as the major component. Although carbohydrate content of pectoralis muscles is significantly hig-

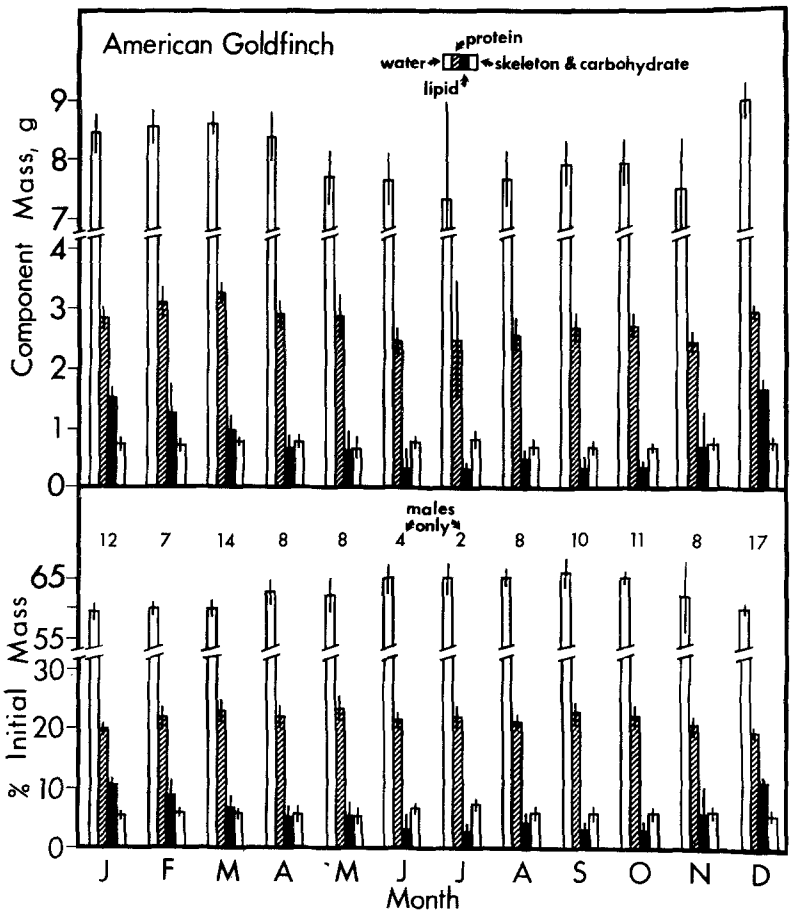

Fig. 2. Mean (and 95% confidence intervals) absolute (upper figure) and relative (lower figure) masses of water, lipid, protein, skeleton and carbohydrate in American goldfinches captured in various months of the year. Data for June and July pertain to males only. Sexes are combined for other months. Sample sizes for each month are located between the two figures. Data were collected in 1973–1975

her in winter (see below), the size of this general category of body constituents (including skeleton) remains essentially constant over the year (Fig. 2).

Seasonal Variations in Fatty Acid Content of Neutral Lipids

Proportions of fatty acids in neutral lipids of liver, pectoralis muscle, and furcular depots vary substantially not only among tissues at each season but also with each tissue seasonally (Fig. 3). Linoleic acid (18:2) predominates in all tissues at all seasons. Saturated lipids are present in higher proportions in spring and fall than in winter birds (Fig. 3).

Evaluation by Duncan's multiple range test indicates that the proportions of fatty acids do not differ significantly between liver and pectoralis muscle of winter birds. However, the former tissue does contain the only trace of eicosatrienoic acid (20:3) detected in this study. This uniformity does not extend to the fatty acid composition of depot lipids obtained from the furcular region (Fig. 3). Linoleic acid comprises a significantly ($P<0.05$) greater and palmitic (16:0) and stearic (18:0) significantly smaller ($P<0.05$) percentages of depot lipids than of lipids from the other two sites. Additionally, depot lipids contain measurable amounts (6.7%) of docosatrienoic acid (22:3) but apparently lack arachidonic acid (20:4), a compound representing 9.6% and 8.5% of neutral lipids in liver and pectoralis muscle, respectively.

From winter to spring, the proportions of myristic (14:0) and palmitic acids rise significantly ($P<0.05$) and that of linoleic acid declines significantly ($P<0.05$). Livers of spring birds contained significantly ($P<0.05$) smaller proportions of stearic acid (11.9%) than did their pectoralis muscles (15.9%).

The pattern of fatty acid content in tissues of fall birds resembles more closely that in spring rather than in winter birds. The major difference is that linoleic acid is present in higher proportion in fall than in spring in liver and pectoralis muscle. Myristic and palmitic acids represent significantly ($P<0.05$) smaller proportions of the neutral lipids in liver and pectoralis muscle in fall birds than in corresponding tissues of spring birds. In fall birds, the proportions of linoleic and stearic acids differ significantly between liver and pectoralis muscle ($P<0.05$). Unlike the muscle, liver lacks docosatrienoic acid.

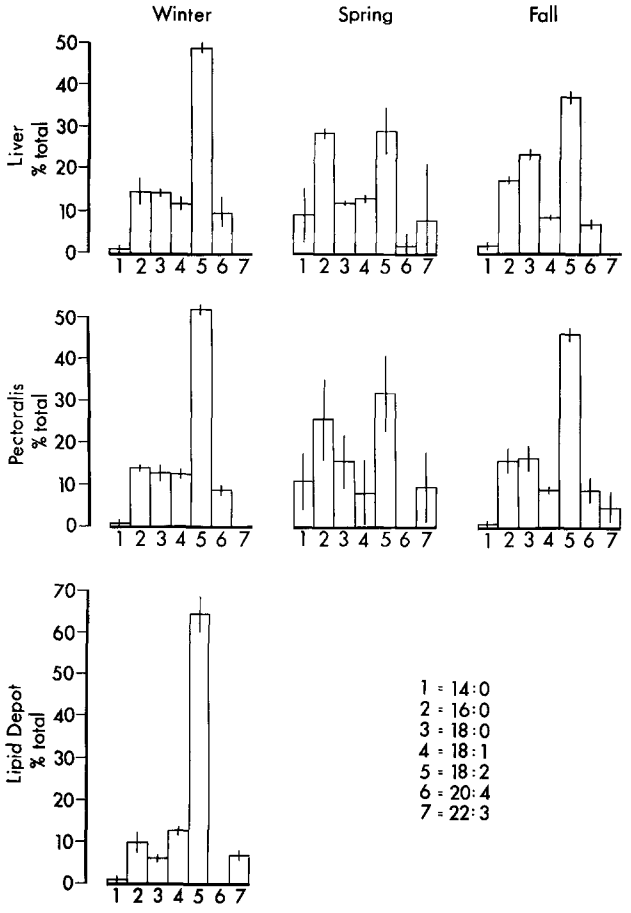

Fig. 3. Mean percentages (and 95% confidence intervals) for fatty acids of neutral lipids in liver, pectoralis muscle, and furcular lipid depot of American goldfinches captured in January (winter), September (fall), and May (spring). Data obtained from four birds at each of the times indicated

Utilization of Substrate during Overnight Exposure to −10 °C

The energetic consequences of cold stress for fasting birds in the inactive phase of their daily cycle and the pattern of substrate utilization, particularly glycogen and fatty acids, were determined through overnight exposures of goldfinches to −10 °C. A statistical approach was necessarily employed, with samples containing different birds providing the data on the condition of goldfinches at the commencement and conclusion of the tests. It should be noted that the energy reserves of the birds tested may be below those with which individuals conclude winter days in nature. Circumstances pertaining to procurement of these birds necessitated trapping them between 0800 and 1300 h. After arrival in the laboratory they were provided with food *ad libitum*. However, they did not always fully avail themselves of it, doubtless because of the unfamiliarity of their new surroundings.

As would be anticipated, prolonged fasting at −10 °C produces a substantial decline in total body mass, experimental birds at the end of tests weighing on the average 1.27 g, or 8.9% less than control individuals (Table 3). This reflects the significant ($P < 0.001$) difference in neutral lipid between the two groups, similarly averaging 1.27 g. While the decline in lipid appears primarily to affect fat depots, it also seems to reflect changes in individual organs. For example, liver mass undergoes a significant decline ($P < 0.0001$). This only amounts to 0.06 g, but it is disproportionately high, representing 16.2% of initial liver mass. The pectoralis muscle mass also responds

Table 3. Mean body masses and body constituents of fasting goldfinches exposed to −10 °C overnight for 17 h. Sample sizes are indicated below each mean

Component	Control[a] birds (1)	Experimental birds (2)	Prob. 2<1
Body mass g	14.33± 0.176 12	13.06± 0.213 12	0.003
Liver mass g	0.37± 0.013 11	0.31± 0.003 11	0.0001
Pectoralis muscle mass[b] g	1.10±0.029 11	1.02±0.036 11	0.001
Total body lipid g	1.79± 0.058 6	0.52± 0.128 6	0.0001
Total body protein[c] g	2.67± 0.066 6	2.56± 0.056 6	N.S.
Total body water g	8.51± 0.167 6	8.30± 0.188 6	N.S.
Liver carbohydrate µg or (µg/g liver)	185.33±34.623 (500.42±78.417) 6	118.00±26.481 (389.23±86.433) 6	N.S.
Pectoralis muscle carbohydrate[d] mg or (mg/g muscle)	1.30± 0.199 (0.56± 0.072) 6	1.66± 0.364 (0.79± 0.178) 6	N.S.
Pectoralis muscle fatty acids µmoles	26.54± 2.675 6	19.90± 3.346 6	N.S.

[a] Control birds were sacrificed without being exposed to −10 °C
[b] Value represents mass of pectoralis muscle from one side
[c] Value includes protein of flight feathers but not contour feathers
[d] Value represents a measurement of both pectoralis muscles

Table 4. Mean (±S.E.M.) percentages of various fatty acids in the pectoral muscles of fasting goldfinches held overnight for 17 h at −10 °C. Sample sizes equaled six for all means

Treatment	Fatty acid							
	14:0	16:0	18:0	18:1	18:2	20:4	22:3	22:4
Control[a]	1.9±0.37	11.5±1.45	10.1±1.83	11.5±1.02	32.7±4.97	4.1±1.78	29.4±9.46	0.7±0.70
Experimental	1.2±0.59	12.7±1.58	12.0±2.16	13.1±0.89	33.4±4.65	5.0±1.45	20.9±7.79	1.2±0.88

[a] Control birds were sacrificed without having been exposed to −10 °C

Table 5. Mean (±S.E.M.) body mass and various body constituents of goldfinches exposed to −70 °C during summer and winter. Sample sizes are indicated below each mean

Component	Summer		Winter	
	Control[a]	Experimental	Control[a]	Experimental
Body mass g	11.67 ± 0.163 24	11.25 ± 0.491 9	14.16 ± 0.235 12	12.83 ± 0.340 4
Total body lipid g	0.395± 0.045 24	0.390± 0.061 9	1.52 ± 0.098 12	0.69 ± 0.109 4
Liver carbohydrate μg or (μg/g liver)	48.87 ± 4.741 (205.39 ±18.080) 8	59.63 ± 7.510 (263.98 ±42.346) 9	140.63 ± 46.493 (491.29 ±165.777) 8	161.00 ± 44.000 (550.26 ±163.550) 5
Pectoralis muscle carbohydrate[b] mg or (mg/g muscle)	1.750± 0.404 (0.84 ± 0.189) 8	1.139± 0.266 (0.562± 0.123) 9	4.364± 0.847 (2.068± 0.341) 8	0.942± 0.585 (0.444± 0.265) 5

[a] Control birds were sacrificed without having been exposed to −70 °C
[b] Value represents total for both pectoralis muscles

significantly to the level of cold stress employed in this particular set of tests. This tissue averages 0.08 g less (7.3% of initial pectoralis mass) in experimental birds at the end of cold stress than in controls. Amounts of total body protein, water, and pectoralis and liver carbohydrates do not differ significantly between control and experimental goldfinches. The amounts of carbohydrate stores in liver and pectoralis muscle, respectively, likewise do not differ significantly between control and experimental birds (Table 3).

Overnight Utilization of Fatty Acids at −10 °C

The fatty acid components of the neutral lipids of control and experimental goldfinches were analyzed in connection with 17-h tests at −10 °C. The proportions of these compounds do not differ significantly between the two groups (Table 4). The mean amounts of fatty acids present in the pectoralis muscles of control and experimental birds are 26.5±2.67 and 19.8±3.35 μmoles, respectively. These figures do not differ significantly. The control birds, which were captured in the morning and held undisturbed with food until 1700 h (Table 4), did differ from birds sacrificed within 1 h after capture (Fig. 3) in showing docosatetraenoic (22:4) and docosatrienoic (22:3) acids in the pectoralis muscles. Whether this reflects a selective depletion associated with struggling in newly captured individuals, or a replenishment or initial acquisition of 22:3 and 22:4 acids from the laboratory diet in the control birds is unknown.

Utilization of Substrate under Severe Cold Stress

The use of lipid and carbohydrate as substrates for thermogenesis during short-term severe cold stress was estimated from comparison of experimental birds at conclusion of −70 °C tests and controls (Table 5). Total body mass and body lipid do not differ significantly between the control and experimental birds tested in summer. However, in the winter when the birds are able to operate at −70 °C for 4–8 h, both body mass and lipids are significantly ($P<0.01$) lower in the experimental group.

Mean amounts of carbohydrates in liver and pec-

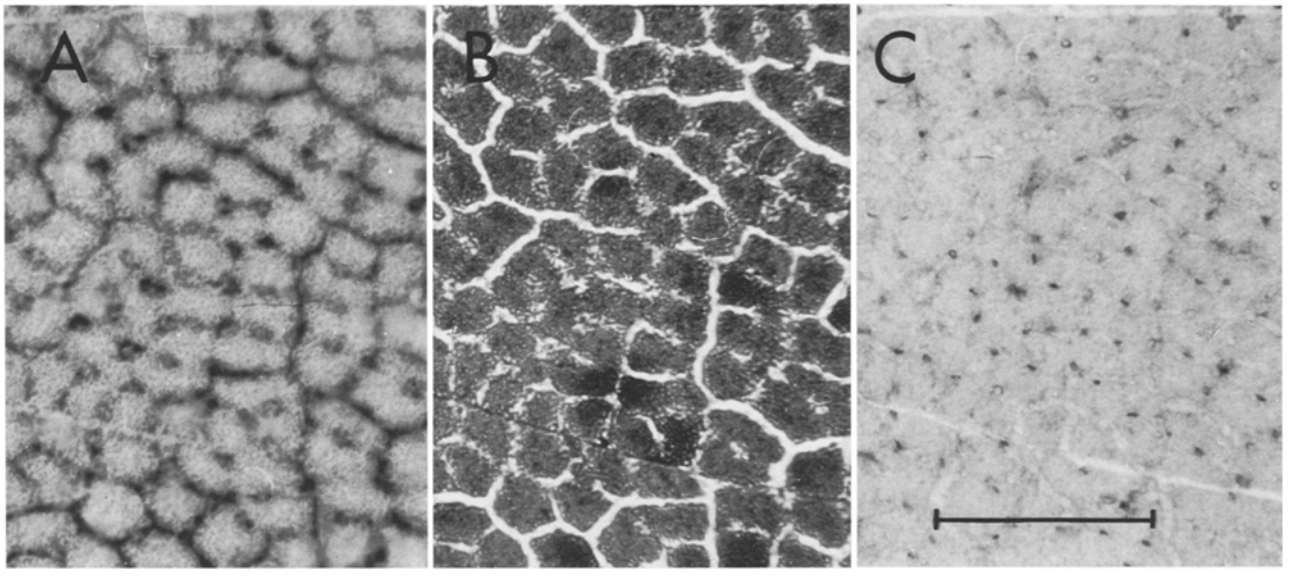

Fig. 4A–C. Photomicrographs (magnification ×384) of serial cross-sections of the pectoralis muscle of American goldfinches. The sections were stained for activities of succinic dehydrogenase (**A**), myofibrillar ATPase (**B**), and capillary membrane phosphatase (**C**). The scales are identical in each panel and the line in panel "**C**" denotes 100 μm

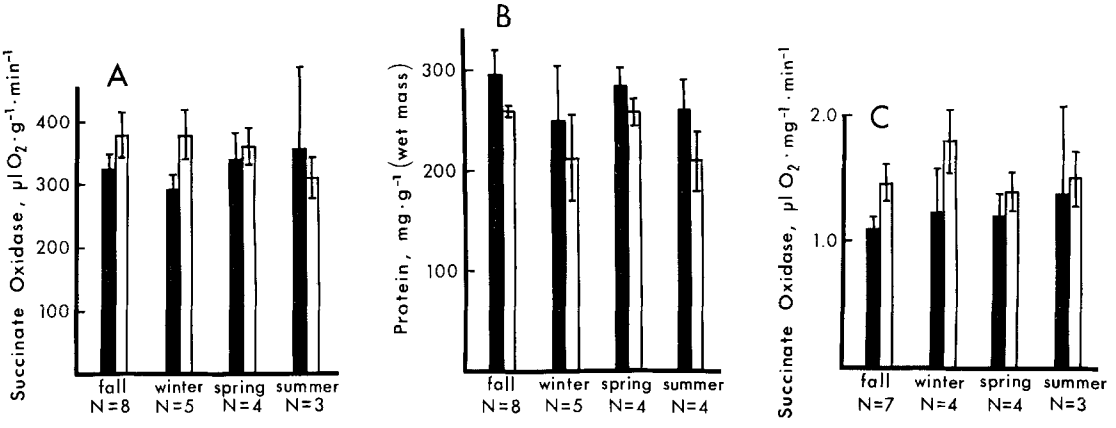

Fig. 5A–C. Mean (and 95% confidence intervals) succinate oxidase activities of heart (unshaded bars) and pectoralis muscles (shaded bars) of American goldfinches captured in October (fall), January (winter), April (spring), and July (summer). **A** Succinate oxidase activity per g of homogenate; **B** Protein content per g of homogenate; **C** Succinate oxidase activity per mg of homogenate protein

toralis muscle did not change significantly during the short exposure to −70 °C that summer birds tolerate (Table 5). Evidently, no sizeable depletion of carbohydrates occurs in this tissue during brief exposure to severe cold. Levels of liver carbohydrate were not altered significantly during 4–8 h at −70 °C tolerated by goldfinches in winter. However, the mean control level of pectoralis muscle carbohydrate (4.36 ± 0.85 mg) is significantly ($P < 0.01$) higher than that for experimental birds at the conclusion of the test (0.94 ± 0.58 mg). This suggests significant depletion of carbohydrate in the pectoralis muscles during exposure to severe cold stress. With respect to this aspect of the performance of winter birds, it is pertinent that their pectoralis muscles contain significantly higher initial concentrations of carbohydrates than those of summer individuals (Table 5). However, the primary dependence of goldfinches on body lipids during cold stress is indicated by the fact that neutral lipids and carbohydrates differ on the average by 700 mg and 3 mg, respectively, between experimental individuals tested at −70 °C and controls.

Histochemistry and Oxidative Capacities of Pectoralis Muscles

All fibers of pectoralis muscles of goldfinches show high succinic dehydrogenase and high myofibrillar ATPase activities (Fig. 4) and thus are similar to the fast twitch, high oxidative fibers in skeletal muscles of mammals (Maxwell et al., 1973, 1977). The histochemical profile of the pectoralis muscle fibers does not vary seasonally. Mean fiber cross sectional area does not differ significantly between summer and winter birds. The pooled mean fiber area is $560 \pm 24\ \mu m^2$. Pectoralis muscle fibers average 5.12 ± 0.14 adjacent capillaries, or 4150 ± 640 capillaries per mm^2. The average capillary to fiber ratio (capillaries mm^{-2}: fibers mm^{-2}) is 2.02 ± 0.06. Succinate oxidase activity of homogenates of pectoralis muscle from fall, winter or spring birds did not differ significantly from values for summer birds whether activity was expressed per g of muscle wet mass or per mg of homogenate protein (Fig. 5A, 5C). Succinate oxidase activity of homogenates per g of wet mass of heart muscle was lower in summer birds than in individuals at any other season (Fig. 5A). However, seasonal variation in protein content of homogenates occurred (Fig. 5B). When activity is expressed per mg of homogenate protein, only the activity of homogenates of heart muscle of winter birds is significantly greater than the summer value (Fig. 5C).

Discussion

Introduction

Our initial study (Dawson and Carey, 1976) established that adjustment to winter cold by American goldfinches principally involves a form of metabolic acclimatization, as noted in the introduction to this report. Seasonal alterations in plumage insulation do occur over the year in goldfinches; however, no direct correspondence exists between their timing and seasonal changes in thermogenic capacity involved in this metabolic acclimatization. The data presented here allow us in ensuing sections of this discussion to examine two questions pertaining to this example of seasonal acclimatization: 1) what changes occur seasonally in body mass and composition and in oxidative capacity of skeletal muscle; and 2) what patterns of substrate utilization are evident in goldfinches under moderate and severe cold stress at different seasons? This examination should facilitate identification of the factors responsible for superior thermogenic capacities of winter goldfinches.

Seasonal Changes in Body Mass and Composition

One of the more conspicuous aspects of seasonal differences in American goldfinches in southern Michigan involves a 32% rise in body mass between July and December-January (Fig. 1, Table 1). The annual variation in body mass observed for American goldfinches in the Ann Arbor area differs from that evident in birds of this species near Cincinnati, Ohio (Wiseman, 1975). Our birds were 1–2 g lighter and their winter peak of body mass occurred approximately a month earlier than in the Ohio birds. The difference in body masses probably results from the fact that the latter birds were predominantly collected late in the day when fat reserves were fully replenished and crops full of seed, whereas ours were mostly obtained before noon. Despite the differences in absolute body mass, the Ohio goldfinches resembled Michigan individuals in being approximately one-third heavier in winter than in late spring-early summer. The difference in time of attainment of the maximal mean masses for Michigan birds (December-January) and Ohio birds (February) is less readily explicable. However, a difference of this type has been noted between Alaskan and European populations of common redpolls (*Carduelis* [*Acanthis*] *flammea*) (Pohl and West, 1976). Such population differences in redpolls and in goldfinches could be linked to differences in local thermal regimes, daily energetic requirements, nutritional factors, or in endogenous circannual rhythms of fattening.

Other cardueline finches, particularly those wintering in northern latitudes, also exhibit hibernal increases of body mass (Partin, 1933; Bartleson and Jensen, 1955; Abs, 1964; Newton, 1969; Pohl and West, 1976; White and West, 1977), although this pattern may not be universal (King and Wales, 1965). This increase in body mass appears linked either proximately or ultimately to cold because it is inversely correlated with ambient temperatures, usually below some threshold, and because noticeable increases in body mass or total body lipid are not commonly associated with migration in cardueline finches (Bartleson and Jensen, 1955; Evans, 1969b; Eriksson, 1970; Wiseman, 1975; White and West, 1977).

Measurement of individual body constituents in birds reveals that seasonal patterns of variation of body mass related to migration or winter acclimatization are associated with variation in neutral lipid content, although total protein and water may also vary (Zimmerman, 1965; Johnston, 1966; King and Farner, 1966; Helms et al., 1967; Evans, 1969a, 1969b; Newton, 1969; Barnett, 1970; Morton et al., 1973; Chaplin, 1974; White and West, 1977). The amount of lipid stored by migratory birds can be substantial,

varying according to the total length of flight to be undertaken and the rapidity of migration (Odum et al., 1961; King et al., 1963). Daily lipid storage by birds during winter is more modest, appearing only sufficient to supply energetic demands overnight and into the following day. This latter pattern apparently results from a precisely controlled process of energy storage rather than some absolute limit on lipid deposition, for representatives of several of these species deposit up to twice as much lipid prior to migration as they do during winter (King, 1972; Blem, 1976). Adult American goldfinches contain an average of 1.67 g of neutral lipid in December (Table 2). Were all this mobilizable in the cold (see below), it would, at 9.3 kcal/g (38.9 kJ/g), represent an energy store of 15.6 kcal (65.3 kJ), an amount sufficient to provide maintenance of a metabolic rate twice BMR (Dawson and Carey, 1976) for 23 h in 15.1-g December birds. Such a rate would permit temperature regulation at a T_a of approximately -10 °C (Dawson and Carey, 1976). Our analysis of body composition was largely based on goldfinches captured before noon. Therefore, the mean values for neutral lipid presented in Table 2 probably represent underestimates of the fat stores with which winter birds begin their nightly fast. The figure of 23 h for maintenance of twice-standard rates of heat production appears conservative for both this reason and the fact that it neglects food material present in the crop at the end of the daily feeding period.

Seasonal differences exist in goldfinches not only in the amount of lipid stored but also in the kinds of fatty acids in the tissue lipids (Fig. 3). The primary seasonal differences reflect the predominance of linoleic acid (18:2) in winter and the greater proportion of saturated fatty acids (myristic, 14:0; palmitic, 16:0, and stearic, 18:0) in spring and fall. The predominance of linoleic acid in other avian tissues, especially in winter, has been noted in bobolinks, *Dolichonyx oryzivorus*, (Walker, 1964); juncos, *Junco hyemalis* (Bower and Helms, 1968); ptarmigan, *Lagopus lagopus* (West and Meng, 1968a); redpolls, *Carduelis flammea* (West and Meng, 1968b); and white-crowned sparrows, *Zonotrichia leucophrys gambelii* (Morton and Liebman, 1974).

The available data on the fatty acid components of tissues of wild birds have recently been summarized by Blem (1976). Seasonal increases in the proportion of unsaturated fatty acids in avian lipid usually coincide with colder ambient temperatures (Bower and Helms, 1968; Barnett, 1970; Palokangas and Vihko, 1972; this study). Perhaps the functional significance of this is related to the fact that the chain length and degree of saturation of the fatty acid molecule may affect its rate of uptake or catabolism, the configuration of the glyceride molecule of which it is a part, or the rate of breakdown of the glyceride molecule. Each of these could influence thermogenic capacity.

American goldfinches contain more protein in winter and spring (December-May) than in the remainder of the year (Fig. 2). This constituent was also elevated in a female collected in July just prior to egg-laying (Table 2). This seasonal pattern differs from that for thermogenic capacity, which declines abruptly in late winter (Dawson and Carey, 1976). The particular tissues in which the increase of protein occurs remain to be determined. Certain seasonal changes that might account for it have been noted in the mass of the digestive tract (Pendergast and Boag, 1973) and in lean dry mass of body and pectoralis muscles (Fry et al., 1970, 1972; Baggott, 1975; Jones and Ward, 1976) of several species of birds.

Total body water of goldfinches also varies substantially, being higher in winter and early spring (December-April) than in the remainder of the year (Fig. 2). The 1.31-g difference separating summer (August) from December adults of both sexes accounts for 41% of the change in body mass (Table 2). With this and previously cited information, the differences in body mass between these sets of birds can be compartmentalized as follows: total body water, 41%; lipid, 38%; protein, 13%; other, 8%. The exact contribution of each of these would be somewhat different were comparisons made between other months, for the change in lipid, protein, and total body water follow different courses. The changes in mass noted between fall and winter bullfinches (*Pyrrhula pyrrhula*), representatives of another carduline species, involve a quite different breakdown: water, 65%; lipid, 12%; lean dry material (mainly protein, excluding feathers), 25% (Newton, 1969).

Oxidative Capacities of Skeletal Muscle

Adult birds evidently lack non-shivering thermogenesis (Hart, 1964; West, 1965). One should therefore ask whether the increased thermogenic capacity of winter goldfinches in severe cold is associated with any enhancement of oxidative capacity of skeletal muscle. We addressed this question by measuring at different seasons succinate oxidase activity of the pectoralis muscles, which must play a prominent role in the shivering thermogenesis of goldfinches. The results obtained (Fig. 5) indicate that oxidative capacity is not increased significantly during winter on either a mass- or protein-specific basis. Oxidative capacity of pectoralis muscles of house sparrows (*Passer*

domesticus) also appeared to remain stable among birds maintained at 1, 23, or 35 °C for 7 weeks (Chaffee and Mayhew, 1964), although the inordinately high values of succinate oxidase activity reported for these birds raise a question as to the validity of this finding. The pectoralis muscles of American goldfinches consist exclusively of high oxidative, fast-twitch fibers (Fig. 4). The ability of such fibers to meet the energetic requirements of sustained flapping flight is related to their high oxidative capacity and the tendency to catabolize free fatty acids preferentially to glycogen (Chandra-Bose and George, 1964; George and Nene, 1965). Evidently, the metabolic machinery necessary to support flight is more than adequate to deal with thermogenic requirements in winter. Flapping flight in goldfinches requires an estimated metabolic expenditure of 4.5 times BMR, whereas 3 times BMR should be sufficient to maintain homeothermy at T_a's down to approximately −30 °C (Dawson and Carey, 1976).

Mobilization and Utilization of Substrates for Thermogenesis Under Moderate and Severe Cold Stress

The annual cycle of lipid deposition closely parallels that of the thermogenic capacities of goldfinches (Dawson and Carey, 1976). The importance of lipid availability to thermogenic capacity is also suggested by other considerations. First, a direct correlation ($r=0.71$; $P<0.05$) exists between the initial body mass of goldfinches tested in January and February and their ability to maintain peak oxygen consumption at −70 °C. Differences in body mass among goldfinches at this season principally reflect changes in lipid content. Secondly, captive goldfinches maintained during winter in outdoor flight cages or indoors at 10 °C, did not deposit as much lipid as wild birds and were as limited in their ability to prevent hypothermia at −70 °C as typical summer or fall birds.

Thermogenic capacity must depend on more than mere seasonal accumulation of lipid. Triglycerides are synthesized in the liver of birds and then transported to adipose tissue, where apparently the fatty acids are removed from the glyceride molecule and then reesterified into triglyceride for storage (Goodridge and Ball, 1967; O'Hea and Leveille, 1969). Mobilization of fatty acids from lipid depots is a multistep process involving numerous hormones, intracellular messengers, and enzymes (Goodridge and Ball, 1965; Goodridge, 1975). Since avian muscles are predominantly dependent upon free fatty acids supplied from lipid depots by the blood rather than from intracellular or intramuscular stores (Vallyathan and George, 1969; Vallyathan et al., 1970), the lipid depots must be highly responsive to metabolic requirements. In this connection it is of interest that goldfinches tested at −70 °C in summer did not mobilize significant amounts of lipid before becoming hypothermic, whereas winter individuals utilized an average of 0.8 g in such tests (Table 5). It is important to note that winter goldfinches ultimately became hypothermic at −70 °C even though approximately 0.7 g of neutral lipid remained. Perhaps some hormonal or enzymatic factor or other substrate such as glycogen (see below) became depleted in advance of the exhaustion of lipid supplies, at the very high metabolic rate required at −70 °C. Kendeigh (1945) also found that birds starved at lower (−39 to +20 °C) temperatures contained more lipid at death than those of birds starved at higher (21 to 35 °C) ones. All this information suggests to us that an increased ability to mobilize lipid rapidly may be a primary determinant of the enhanced thermogenic capacities of winter goldfinches. Such a change could involve sensitization of adipose tissue to hormonal stimuli, or increases in concentrations of circulating hormones or depot enzymes.

Although free fatty acids are clearly the major substrate for sustained muscle contraction of avian (George and Vallyathan, 1964) and mammalian skeletal muscle (Issekutz et al., 1970; Therriault et al., 1973; Minaire et al., 1973; Paul and Holmes, 1975), these compounds provide only 77% of the energy requirement in avian muscle (George and Jyoti, 1957) or 78% of the energy expenditure during thermogenesis in dogs (Minaire et al., 1973). The remaining energy must be supplied by catabolism of glycerol, amino acids, or glycogen.

The metabolic fate of glycerol in birds is currently undefined. Amino acids are released from mammalian muscle during contraction and can be used by the liver as a substrate for gluconeogenesis (Felig and Wahren, 1971). It is well known that muscle protein is utilized as an energy source during starvation in birds, but our data on total body protein do not permit estimation of the extent of contribution of muscle protein as a component of the total energy expenditure during thermogenesis.

Liver and muscle stores of glycogen appear essential for mammalian muscles utilizing lipid during sustained shivering or exercise (Pernod et al., 1972; Minaire et al., 1973), despite the indication that high levels of citrate produced during catabolism of free fatty acids can markedly inhibit glycolysis (Newsholme and Start, 1973). Although the pectoralis muscles of goldfinches consist of fibers that primarily metabolize lipid, glycogen is deposited in those mus-

cles (Table 3 and 5) and must be used as a substrate by them, for glucose-6-phosphatase appears absent from avian muscle (Hazelwood, 1972). That glycogen utilization may play an important role in seasonal enhancement of thermogenesis is suggested by the fact that goldfinches deposit significantly ($P<0.01$) more glycogen in the pectoralis muscles in winter than in summer (Table 5).

Values for liver glycogen of goldfinches are substantially lower than those reported for other birds (Hazelwood and Lorenz, 1959; Naik, 1963; Migliorini et al., 1973). It is unclear whether this difference results from the stress of captivity and handling, or interspecific differences. The concentrations of liver and muscle glycogen do not appear to change significantly during moderate cold stress at −10 °C (Table 3). Such stability may involve a balance between glycolysis and gluconeogenesis rather than a lack of turnover of carbohydrate. Muscle carbohydrate in winter was significantly lower in experimental birds following severe cold stress at −70 °C than in control individuals (Table 5). We do not know if the reduction of carbohydrate suggested by this result occurred continuously over the course of the 6–8 h test or abruptly at the end. In the latter case, the decline in availability of carbohydrate might well have precipitated the drop in maximal metabolic rate and hypothermia.

Conclusion

Many uncertainties remain concerning the factors responsible for the enhanced thermogenic capacities of American goldfinches in winter. However, we can now specify that this enhancement depends on increased supplies of fat and muscle glycogen available to these birds rather than to any increased oxidative capacity of the pectoralis muscles. Our study also provides indications that heightened abilities to mobilize and utilize these substrates may be of particular importance to the increased abilities of winter birds to sustain high rates of heat production. The analysis of winter acclimatization in the American goldfinch should now turn to an examination of seasonal changes in these processes.

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